Journal of Advances in Medicine and Medical Research

26(8): 1-7, 2018; Article no.JAMMR.41632
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Occurrence and Detection of bla_{KPC} Producing *Klebsiella pneumoniae* in an Indian Tertiary Hospital

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Authors' contributions

This work was carried out in collaboration between all authors. Author AB designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors DKK and MS managed the analyses of the study. Author SB managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2018/41632
Editor(s):
(1) Rodrigo Crespo Mosca, Department of Biotechnology, Institute of Energetic and Nuclear Research (IPEN-CNEN), University of Sao Paulo (USP), Brazil.
Reviewers:
(1) Marilene Rodrigues Chang, Federal University of Mato Grosso do Sul, Brazil.
(2) Victoire Gadou, Felix-Houphouet-Boigny University, Côte D'Ivoire.
Complete Peer review History: http://www.sciencedomain.org/review-history/24957

Original Research Article

Received 18th March 2018
Accepted 30th May 2018
Published 4th June 2018

ABSTRACT

Background: *Klebsiella pneumoniae* is one of the most important gram-negative bacterial pathogens which has caused worldwide concern because of its association with life threatening nosocomial infections and its multidrug-resistant (MDR) property. As a result of the increased use of antibiotics; Carbapenemases are widespread in Enterobacteriaceae family and particularly in *K. pneumoniae* so effective treatment options are decreasing. The aim of this study was to detect *bla*KPC gene in *Klebsiella pneumoniae* by conventional PCR and to compare the phenotypic, ie Modified hodge test and genotypic methods ie PCR for detection of KPC carbapenemases of the recovered isolates of *Klebsiella pneumoniae*.
Study Design: Cross Sectional Prospective.
Place and Duration of Study: This study was conducted in the Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences, Srinagar; in India over a period of one year and three months (june 2014-september 2015).
Methods: This study included all isolates of *Klebsiella pneumoniae* recovered from blood culture of

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the patients. The identification and antimicrobial susceptibility were done on Vitek 2 compact. Isolates that were resistant to meropenem were included for phenotypic (Modified Hodge Test) and genotypic (Conventional Polymerase Chain Reaction) tests.

Results: A total of 70 *Klebsiella pneumoniae* isolates were recovered. Out of these 55 were meropenem resistant and 15 were sensitive. 76.3% isolates were MHT positive and 23.7% were negative. blaKPC gene was present in 64.5% and absent in 34.5% isolates. Out of 42 MHT positive isolates 81% were carrying *bla*KPC gene.

Conclusion: It was seen that a higher prevalence of *bla*KPC producing *Klebsiella pneumoniae* were isolated from patients admitted in ICU (intensive care unit). So formulation of an antimicrobial policy and its strict implementation is needed along with appropriate infection control measures to curtail its emergence and spread.

Keywords: Klebsiella pneumonia; carbapenem resistance; modified hodge test; polymerase chain reaction; blaKPC gene.

1. INTRODUCTION

Klebsiella pneumoniae is largely thought of as an opportunistic pathogen, but the emergence of hypervirulent strains over the past decade have demonstrated the capacity to infect otherwise healthy individuals and is associated with life threatening nosocomial infections [1,2]. It is an *Enterobacteriaceae* member which often displays resistance towards β-lactam antibiotics, particularly through β-lactamase expression of which the most important are cephalosporinases, such as extended-spectrum β-lactamases (ESBLs) and carbapenemases [3].

Most prevalent carbapenemases are the KPC carbapenemases, found mostly on plasmids in *Klebsiella pneumonia* [4]. First member of the KPC family was discovered through the ICARE surveillance project in a *K. pneumoniae* clinical isolate from North Carolina in 1996 [5].

The gene encoding the KPC enzyme is usually flanked by transposon-related sequences and has been identified on conjugative plasmids, therefore, potential for dissemination is significant [6,7,8]. Isolates that acquire this enzyme are usually resistant to several other classes of antimicrobial agents used as treatment options. So laboratory identification of KPC-producing clinical isolates will be critical for limiting the spread of this resistance mechanism [9].

Misidentification of KPC-producing bacteria is common with standard susceptibility testing. It has been reported that automated systems will identify 7-87% of KPC-producing *K. pneumoniae* as susceptible to imipenem or meropenem [9]. The great variability that has been observed in carbapenem minimal inhibitory concentrations (MICs) by routine testing is likely related to the phenotypic heterogeneity among isolates, giving the appearance of susceptibility *in vitro* [10].

In order to reduce and control the further spread of carbapenem resistance, rapid identification is crucial so that appropriate treatment can be applied [11]. Classical microbiological methods often give slow results only after additional cultivation for 24 or even 48 h [12,13]. A variety of molecular methods have been developed, such as PCR-based methods which are a lot quicker and can give results within a few hours. So the present study was done to detect KPC carbapenemase and know the prevalence of *bla*KPC gene in meropenem resistant *K. pneumoniae* isolates by conventional polymerase chain reaction.

2. MATERIALS AND METHODS

2.1 Study Design

This study was conducted in the Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences, Srinagar in India; over a period of one year and three months (June 2014-september 2015).

2.2 Methodology

The study included all isolates of *Klebsiella pneumoniae* recovered from blood culture of the patients. The identification and antimicrobial susceptibility of the isolates was done on Vitek 2 compact (bioMe'rieux, Inc., France). Isolates resistant to meropenem were included for phenotypic and genotypic testing of KPC. Modified Hodge test was done to detect the carbapenemase production in these isolates.

These isolates were then tested for *bla*KPC gene by conventional polymerase chain reaction.

2.3 Modified Hodge Test

Carbapenemase production was detected by Modified Hodge test.

2.3.1 Principle

The test isolate produces the enzyme and allows growth of a carbapenem susceptible strain (*E. coli* ATCC 25922) towards a carbapenem disk.

2.3.2 Procedure

Modified Hodge testing was performed as previously described (CLSI, 2014) [14].

2.3.3 Quality Control

***Positive Control*:** Carbapenemase producing *Klebsiella pneumoniae* strain BAA-1705 (Microbiologics, St. cloud USA).

***Negative Control*:** Carbapenemase negative *Klebsiella pneumoniae* strain BAA-1706. (Microbiologics, St.cloud USA).

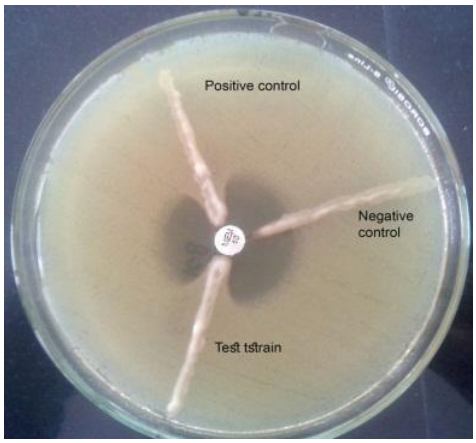

Fig. 1. Modified hodge test (positive result) for carbapenemase detection

2.4 Polymerase Chain Reaction

DNA Extraction: Molecular identification of KPC-producing *Klebsiella pneumoniae* was performed by *bla*KPC PCR using bacterial lysates from overnight broths prepared by removal of 200 µl of broth culture, centrifugation (12,000 × *g*; 2 min), resuspension in 200 µl of molecular-grade water, boiling at 95°C for 10 min, and discarding the cellular debris by centrifugation (12,000 × *g*; 2 min at 4°C). PCR analysis for *bla*KPC was performed with 1 µl of cell lysates, using the following primers designed to identify all *bla*KPC genes (bla_{KPC-1} through bla_{KPC-7}): KPC forward (ATGTCACTGTATCGCCGTCT). KPC reverse (TTTTCAGAGCCTTACTGCCC). The Reaction was set up in a PCR vial, after adding the master mix, the forward and reverse primers and the extracted DNA. 25 µl of Master Mix contained 10X Taq buffer, 2mM Mgcl2, 0.4 mM dNTPs mix, and 2U *Proofreading* Taq DNA polymerase. (Thermo SCIENTIFIC ,USA) Lysates derived from *Escherichia coli* ATCC 25922 and *bla*KPC carrying *K. pneumoniae* strain 1705 _were used as negative and positive controls, respectively, in each PCR. The PCR conditions were as follows: 15 min at 95°C and 38 cycles of 1 min at 94°C, 1 min at 62°C, and 1 min at 72°C, followed by an extension step of 10 min at 72°C [15]. The PCR products were subjected to electrophoresis on 2% agarose gel stained with ehidium bromide and visualized with UV light. The blaKPC gene gave band at 893bp. Fig. 2.

3. RESULTS

A total of 70 non duplicate *Klebsiella pneumoniae* were isolated from patients admitted or attending the out patient department (OPD). Out of these isolates 55(78.5%) were meropenem resistant and 15(21.5%) were meropenem sensitive. Most of the isolates were recovered from specimens obtained from ICU patients, (54; 77%), followed by patients admitted in inpatient department (IPD), (14; 20%) and least from patients attending OPD (2; 3%)". Among the isolates recovered from ICU patients, 44(81.4%) were meropenem resistant and 10(18.6%) were sensitive, in IPD patients 10(71.4%) were resistant and 4(28.6%) were sensitive, whereas in OPD patients 1(50%) isolate was sensitive and 1(50%) was resistant to meropenem.

The antibiotic susceptibility. pattern of the isolates is tabulated in [Table-1].

Relationship between PCR and MHT is shown in Table 2.

Taking PCR as gold standard, sensitivity and specificity of MHT was 94.4% and 57.8% respectively.

Table 1. Antimicrobial susceptibility pattern of *Klebsiella pneumoniae* isolates on Vitek 2

Antibiotic	Sensitive no. (%)	Resistant no. (%)
Colistin	70(100)	0(0)
Tigecycline	60(85.5)	10(14.5)
Meropenem	15(21.5)	55(78.5)
Ertapenem	13(18.5)	57(81.5)
Imipenem	14(20)	56(80)
Ceftazidime	14(20)	56(80)
Cefepime	12(17.1)	58(82.9)
Ceftriaxone	9(12.80)	61(87.2)
Cefuroxime	6(8.5)	64(91.5)
Ciprofloxacin	12(17.1)	58(82.9)
Levofloxacin	15(21.4)	55(78.6)
Amoxiclav	13(18.5)	57(81.5)
Pipracillin tazobactam	14(20)	56(80)
Amikacin	14(20)	56(20)
Gentamicin	32(45.7)	38(54.3)
Cotrimoxazole	17(24.2)	53(75.8)

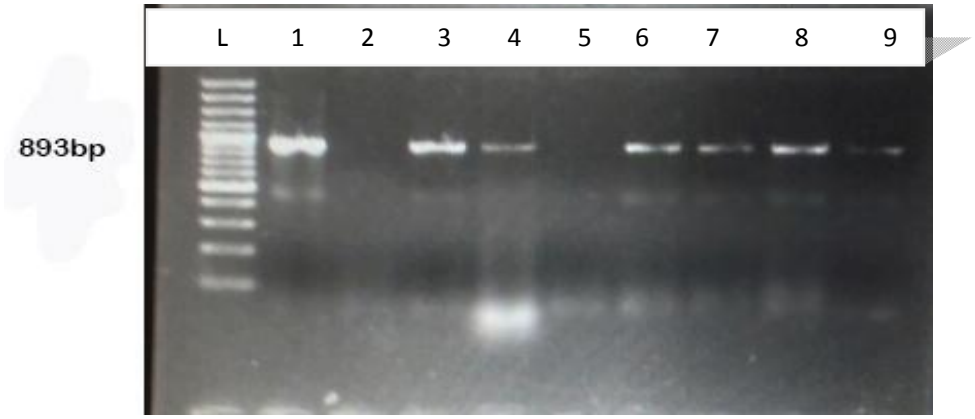

Fig. 2. Poiymerase chain reaction (PCR) for blaKPC gene: Lane-L 100 bp DNA ladder, Lane-1, Positive Control; Lane-2, Negative Control; Lanes 3-9, Test strains

Table 2. Relationship between detection of the blaKPC gene and the results of the Hodge test modified (MHT) in *Klebsiella pneumoniae* isolates

	PCR positive no. (%)	PCR negative no. (%)	Total
MHT positive	34(81)	8(19)	42
MHT negative	2(15.3)	11(84.7)	13

4. DISCUSSION

Multidrug resistant gram-negative bacilli are frequently associated with infections in the patients admitted to intensive care units of hospitals. *Klebsiella pneumoniae* has been identified as one of the most frequent causes of outbreaks reported in neonatal intensive care units (NICUs). It is a known cause of sepsis and had been reported in other studies as the commonest blood culture isolates [16]. In the present study most of the isolates were recovered from specimens obtained from ICU patients ie 54 (77%), followed by patients admitted in IPD 14 (20%) and least from patients attending OPD 2(3%).

In this study isolates exhibited a high degree of resistance to beta-lactams including penicillins and cephalosporins.

Out of 70 isolates tested 91.5% were resistant to cefuroxime, 87.2% to ceftriaxone, 82.9% to cefepime, 80% to ceftazidime, 81.5% to

amoxicillin-clavulanic acid and 80% to piperacillin tazobactam. Among quinolones 82.9% were resistant to ciprofloxacin and 78.6% resistant to levofloxacin and in aminoglycosides 80% were resistant to amikacin and 54.3% to gentamicin. All isolates were sensitive to colistin.

These results in our study are in accordance with study conducted by Hashemi et al. (2014),who in their study found that antimicrobial susceptibility tests revealed a complete resistance to third-generation cephalosporins and a moderate resistance to gentamicin and fluoroquinolones. Colistin was the most active agent against carbapenem resistant *klebsiella pneumoniae* (CRKP) with 99% of susceptibility [17]. Mona Fattouh et al. in their study found that in addition to meropenem, all KPC producing isolates were found to be highly resistant to third-generation cephalosporins, aminoglycosides and fluoroquinolones. However, 17.4% of KPC positive isolates were found to be resistant to colistin [18]. Also according to Parveen et al. 45 meropenem resistant isolates exhibited high resistant to third and fourth generation cephalosporins, tetracycline, gentamicin, cefoxitin, amikacin. Only 14 (32.5 %) were sensitive to tpolymyxin-B [19].

Comparing the results of MHT and conventional PCR for blaKPC gene, out of 42(76.3%) MHT positive isolates 34(81%) were positive for blaKPC and 8(19%) were negative for this gene. This indicates the presence of a carbapenemase other than KPC Carbapenemase. Whereas out of 13(23.7%) MHT negative isolates 2(15.3%) were positive for blaKPC gene. This variable susceptibility can be by genetic suppression leading to a silenced gene; or dosage of the gene that is dependent on the plasmid copy number [20]. Also, 11(84.7%) were negative for blaKPC, so they may have developed a different resistant mechanism other than carbapenemase production.

Our study results are in accordance with study conducted by Shanmugam P et al. in which (93.4%) isolates were resistant to meropenem, MHT was positive in (82.6%) and *bla*KPC gene was detected in (67.4%) isolates and out of the 38 MHT positive isolates blaKPC gene was detected in 28(73.6%) isolates [21]. According to Raghunathan A et al. with isolates of *K pneumoniae* included in the analysis, 46 (98%) of 47 isolates were positive by the MHT and PCR, and 7 (88%) of 8 isolates were negative by the MHT and PCR, with a strong correlation between the results of the MHT and PCR (*P* =.0001; Fisher exact test) [22]. However, according to a study done by Girgis et al. out of 57 clinical isolates of carbapenem resistant *Klebsiella pneumoniae* tested only (21%) isolates were *bla*KPC gene positive by PCR whereas MHT was positive for all isolates with 100% sensitivity [23]. Sensitivity of MHT while comparing with gold standard PCR was 94.4% and Specificity was 57.8% which is in accordance with other study [21].

5. CONCLUSION

It was seen in this study that a higher prevalence of *bla*KPC producing *Klebsiella pneumoniae* were isolated from blood samples with high degree of antimicrobial resistance mostly from the patients admitted in high dependency units ie ICU. So formulation of an antimicrobial policy and its strict implementation is needed along with appropriate infection control measures to curtail its emergence and spread.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24957